Novel Mechanisms of Resistance to Endocrine Therapy: Genomic and Nongenomic Considerations

Anupama E. Gururaj,[1] Suresh K. Rayala,[1] Ratna K. Vadlamudi,[2] and Rakesh Kumar[1]

Abstract   Selective estrogen receptor (ER) modulators have been the most commonly used neoadjuvant therapy for hormone-dependent breast cancer. However, resistance to endocrine therapy, either inherent or acquired during treatment, presents a major challenge in disease management. The causes of resistance to hormone therapy are not well understood and are the subject of active investigation. It is increasingly clear that decreasing sensitivity of ER-positive breast cancer cells to antiestrogens is caused by several factors. Cross talk between ER and growth factor signaling has emerged as a critical factor in endocrine resistance. Here, we present evidence that receptor tyrosine kinase signaling also plays a role in resistance by controlling the subcellular localization of ER signaling components. Localization of ER in either the nuclear or cytoplasmic compartments has functional implications. Recent work suggests that dynein light chain 1, a recently identified substrate of p21-activated kinase 1, modulates ER transactivation functions through a novel ER coactivator function. Likewise, receptor tyrosine kinase signaling can also alter the expression of ER coregulators such as metastasis-associated antigen 1, leading to hormonal independence. Furthermore, proline-, glutamic acid-, leucine-rich protein 1, an ER coactivator involved in both genomic and nongenomic signaling pathways, is activated by epidermal growth factor receptor and plays a prominent role in resistance to tamoxifen. These recent advances suggest new targeted therapeutic approaches that may lead to either reversion or prevention of endocrine resistance in breast tumors.

Tumors in hormone-sensitive organs, such as the breast, ovaries, prostate, and adrenal and thyroid glands, respond to endocrine therapy, and endocrine therapies for breast cancer have been in use for more than a century. Several new endocrine agents have contributed to the marked reduction in breast cancer mortality that has occurred over the past decade (1). However, a substantial proportion of tumors in patients presenting with localized disease and all tumors in patients with metastatic disease become resistant to endocrine therapies (2, 3). The mechanisms for intrinsic and acquired endocrine resistance are still poorly comprehended and are the focus of active investigation.

Selective estrogen receptor (ER) modulators have been the most frequently used adjuvant therapy for hormone-dependent breast cancers. In most cases of resistance, ER is still present in the resistant tumors and its activity continues to regulate tumor growth. Thus, resistance to selective ER modulators could potentially arise via any of the following mechanisms: ER activation in the absence of estrogen; hypersensitivity of ER to low levels of circulating estrogens; or ER activation, rather than inhibition, by estrogen antagonists. At the molecular level, mechanisms responsible for resistance include ER mutations that result in increased sensitivity to ligand or coactivator recruitment, with a resultant increase in ER activity; increased expression of the coactivator proteins that mediate ER activity; down-regulation of corepressor activity, reducing the inhibitory potential of tamoxifen; and posttranslational modifications resulting in ligand-independent activation of the ER. The posttranslational modifications of ER are triggered mainly by activation of growth factor signaling pathways. Because ER also brings about mitogenic and antiapoptotic effects through direct interaction with key components of several signal transduction pathways (nongenomic signaling), altered activity of these pathways could contribute to resistance (4). By understanding which of these pathways is involved in mediating resistance, we might be able to develop strategies for overcoming or bypassing such resistance.

Cross-Talk between ER and Signal Transduction Pathways in Endocrine Resistance

Functional interactions of ER and its coregulators with pathways activated by growth factor signaling have been described. These cytoplasmic and membrane-mediated signaling events controlled by ER are designated the "nongenomic" activities of ER (5, 6). Nongenomic signaling has been linked to

doi:10.1158/1078-0432.CCR-05-2110

rapid responses to estrogen and generally involves stimulation of the Src kinase, mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase, and protein kinase C pathways in the cytosol (6, 7). Furthermore, ER-activated nongenomic pathways have been shown to modify ER or its coactivators by phosphorylation, resulting in an altered topology of ER and its coregulator proteins and eventually leading to ligand-independent activation or differential responses to selective ER modulators (8, 9). Several groups have shown that this interaction emerges as the dominant pathway when breast cancer cells become hormone resistant (4, 10–12).

In addition to its ligand, ER transactivation functions are also stimulated by receptor tyrosine kinases and/or its downstream signaling components (Fig. 1). One such signaling kinase is p21-activated kinase 1 (Pak1), which otherwise could be activated by Rac or Cdc42 (13, 14) or lipids (15–17). Wang et al. (18) showed that Pak1 phosphorylates ER at Ser305 and promotes its transactivation in ligand-independent phenotypes. Pak1 also has been shown to up-regulate cyclin D_1 expression in an ER-independent, but nuclear factor κB–dependent, manner (19).

Functional dysregulation of specific Pak1 substrates (other than ER) has been implicated in Pak1-associated cancer phenotypes and hormone independence. For example, an elegant study showed that dynein light chain 1 (DLC1), a component of the dynein motor, is a physiologic Pak1-interacting substrate and that Pak1 phosphorylation of DLC1 at Ser88 plays a critical role in supporting the anchorage-independent growth of breast cancer cells *in vitro* and in nude mice (20). DLC1 was dysregulated in most of the breast cancer samples and approximately half the tumors had higher-than-normal expression of both Pak1 and DLC1 (20). Recent work has shown that DLC1 interacts with ER and facilitates ER transactivation functions through a novel coactivator (21); evidence also indicated that DLC1 plays a significant role in tumor cell hypersensitivity to estrogen. DLC1 overexpression increased expression of the ER-regulated cell survival protein Bcl-2 and enhanced recruitment of DLC1-ER complex to ER target gene chromatin. Conversely, DLC1 down-regulation

significantly reduced the ability of estrogen to induce progesterone receptor and cathepsin D expression and compromised both the nuclear accumulation and the transactivation functions of ER, suggesting that DLC1 may have chaperone-like activity in the nuclear translocation of ER (21).

PELP1 in the Biology of Hormone-Responsive Cancers

In recent years, ER coregulatory proteins have been found in both nuclear and cytoplasmic cell compartments, raising the possibility that ER coregulators are involved in the nongenomic effects of estrogen and are subject to regulation by growth factor–initiated pathways. Such effects are best exemplified by proline-, glutamic acid-, and leucine-rich protein 1/modulator of nongenomic activity of ER (PELP1/MNAR), which regulates both nuclear and extranuclear estradiol responses (22, 23).

Discovery of PELP1. Initially, using affinity chromatography, PELP1 was identified as a 160-kDa protein that acts as a phosphotyrosine-independent ligand of the Src homologue 2 domain (24). Using the peptide sequences derived from the purified 160 kDa protein, Vadlamudi et al. (22) screened a HeLa cell cDNA library, identifying two cDNAs of different length, which were named PELP1 and PELP2 to reflect the fact that they are unusually rich in the amino acids proline, glutamic acid, and leucine. Initial studies using the longest clone PELP1 (3.8 kb) resulted in generation of a protein product of 160 kDa and its identification as an ERα coactivator (22). Subsequent studies using ERβ affinity chromatography identified a protein that is identical to PELP1 at the amino acid level but differs slightly in cDNA length (3.4 kb); it therefore was assumed to be a new protein and was named MNAR (23). Both the PELP1 and MNAR gene sequences map to the same chromosomal region, 17p13. PELP1 and MNAR cDNAs have identical sequences except for a single additional 435-bp region in PELP1, along with a few sequence errors that have been detected in the original PELP1 sequence (25). Further analysis of the extra PELP1 cDNA regions suggests that the PELP1 cDNA isolated initially was an immature transcript
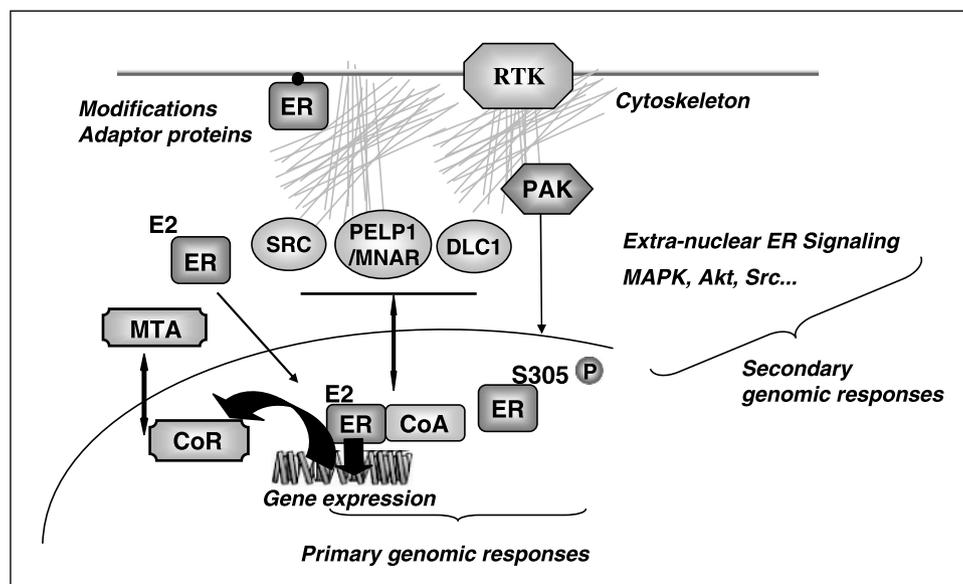
Fig. 1. A simplified model of ER action and regulation by growth factors. Mechanisms of ER activation and downstream events leading to the physiologic effects of ER are indicated, emphasizing both the nuclear and extranuclear activities of ER in breast cancer cells. Estrogen-activated ER translocates into the nucleus to induce gene expression via interaction through its coactivators (*CoA*), thus bringing about its genomic response. ER can also activate cytoplasmic signaling events by association with membrane-bound proteins or other coactivators which eventually bring about nongenomic effects of ER. In the absence of the ligand, ER can be activated by receptor tyrosine kinase (*RTK*) signaling via Pak1 to bring about hormone independence. Corepressors (*CoR*) of ER like those of the MTA family can inhibit ER genomic response by sequestering it in the cytoplasm but amplify the nongenomic effects.

and contained an extra 435-bp intron with consensus splice sites that artificially produced the disparity between the lengths of the PELP1 and MNAR cDNAs. Transfection of PELP1 cDNA results in amazingly efficient splicing of this intron in many cells, resulting in a 3.4-kb mRNA of the 160-kDa protein (25). The ability of PELP1 and MNAR cDNA to generate identical 160-kDa proteins was verified by epitope tagging and reactions with endogenous antibodies (25). The presence of identical amino acid sequences and the perfect match of both cDNAs, including the extra 435-bp intron, to the human genome sequence on chromosome 17p13 suggest that PELP1 and MNAR are identical at the protein level. Thus, it seems that we may be dealing with identical gene products from both PELP1 and MNAR.

Molecular characteristics of PELP1/MNAR. PELP1/MNAR is widely expressed in various tissues, among them the mammary gland, endometrium, ovaries, testis, and brain (22, 26, 27). PELP1/MNAR has been found in both the nuclear and the cytoplasmic compartments, but the proportions of PELP1/MNAR protein that localize in the nucleus and the cytoplasm differ, depending on the tissue (22). PELP1/MNAR contains 10 nuclear receptor–interacting LXXLL motifs; LXXLL motifs 4 and 5 are required for PELP1/MNAR to interact with ER (28). PELP1/MNAR contains two regions rich in cysteine residues, which could possibly form two zinc fingers, and has several consensus sites/motifs. These motifs could potentially interact with proteins containing Src homologue 2, Src homologue 3, and WW domains; for example, the PXXP motif present in the NH_2-terminal region of PELP1/MNAR is implicated in its interaction with Src kinase at the Src homologue 3 domain (28). In addition, PELP1/MNAR is unique in that it possesses an 80-amino-acid domain that efficiently interacts with histones (29). Although PELP1/MNAR has no known enzymatic activity, the presence of multiple protein-protein interaction domains and published evidence suggest that PELP1/MNAR functions as a scaffolding protein, serving as a platform to couple signaling complexes with nuclear receptors such as ER.

PELP1/MNAR in ER genomic actions. PELP1/MNAR functions as a coactivator of both ERα (22, 23) and ERβ (23, 27) and modulates their transactivation functions. In exponentially growing cells, PELP1/MNAR is predominantly localized in the nuclear compartment and associates with both chromatin and nuclear matrix (29). Estrogen stimulation promotes recruitment of PELP1/MNAR to estradiol-responsive gene promoters. PELP1/MNAR efficiently interacts with histones both *in vitro* and *in vivo* (29, 30). PELP1/MNAR associates with ER target gene promoters in a cyclical manner, with recruitment of histone H1 and PELP1 occurring in opposite phases; this behavior suggests that PELP1/MNAR may participate in chromatin remodeling activity by displacing histone H1 in cancer cells (29). PELP1 also interacts with the general transcriptional activator CBP and hormonal stimulation promotes PELP1-associated histone acetyltransferase enzymatic activity (22, 29). Furthermore, PELP1 interacts with corepressors, such as histone deacetylase 2 (30) and metastasis-associated antigen 1 (MTA1; ref. 31), and with other nuclear transcription factors, such as signal transducer and activator of transcription 3 (32). PELP1/MNAR physiologically associates with the cell cycle switch protein pRb in the nuclear compartment and PELP1/MNAR-pRb interactions play a role in the maximal activation of

estradiol target genes such as cyclin D1 (25). PELP1/MNAR nuclear localization, recruitment to the active chromatin, ability to interact with histones and histone-modifying enzymes, and interactions with other nuclear coregulatory proteins all suggest that PELP1/MNAR plays an important role in ER genomic functions.

Role of PELP1/MNAR in nongenomic actions. Evidence also suggests that PELP1/MNAR plays a key role in the ER-mediated generation of nongenomic actions. PELP1/MNAR modulates ER interaction with Src, leading to stimulation of Src enzymatic activity and activation of the extracellular signal–regulated kinase 1 and 2 (23). Studies using ERα, PELP1/MNAR, and Src mutants have shown that coordinated binding of MNAR and ER to the Src homologue 3 and 2 domains, respectively, leads to activation of c-Src and Src-mediated signaling (28). PELP1/MNAR also has the potential to interact with the p85 subunit of phosphatidylinositol-3-kinase, leading to activation of the phosphatidylinositol-3-kinase/Akt (protein kinase B) pathway (33). Growth factor signals induce PELP1/MNAR association with epidermal growth factor receptor, resulting in the tyrosine phosphorylation of the former by the latter (32). Growth factor signals also promote PELP1/MNAR interactions with signal transducer and activator of transcription 3, and these interactions play a mechanistic role in the positive regulation of signal transducer and activator of transcription 3 phosphorylation at Ser727 by activation of the Src-MAPK pathway (Fig. 2; ref. 32). PELP1/MNAR was shown to regulate meiosis by enhancing testosterone-triggered maturation and activation of MAPK by means of its interactions with G proteins (34). New evidence suggests that PELP1/MNAR participates in the androgen receptor–mediated nongenotropic signaling that correlates with the transition of LNCaP human prostate cancer cells to androgen independence (35). The findings that PELP1/MNAR interacts with several growth factor signaling components and cytosolic kinases and that it is able to interact with ER highlight a novel role for PELP1/MNAR in ER nongenomic functions. Such regulatory effects of PELP1/MNAR may have important functional implications in the cross talk between ER and signal transduction pathways.

PELP1/MNAR in Endocrine Therapy Resistance

PELP1/MNAR is widely expressed in breast cancer cells and its expression is deregulated in breast tumors (22, 33). PELP1/MNAR is an ER target gene and its expression is differentially regulated by selective ER modulators depending on the cell line (31). Although PELP1/MNAR is predominantly localized in the nucleus in hormonally responsive tissues (22, 29), in 58% of PELP1/MNAR–positive tumors PELP1/MNAR was localized in the cytoplasm either exclusively or in addition to nuclear localization (33). Model cells that mimic PELP1/MNAR cytoplasmic localization in tumors (PELP1-cyto cells) were hypersensitive to estrogen but resistant to tamoxifen. PELP1-cyto cells, but not parental MCF-7 cells, formed xenograft tumors in nude mice (33). In addition, relative to MCF-7 cells, PELP1-cyto cells exhibited increased association of PELP1/MNAR with Src, enhanced MAPK activation, and constitutive activation of Akt. The altered localization of PELP1/MNAR to the cytoplasm was sufficient to trigger its interaction with the p85 subunit of
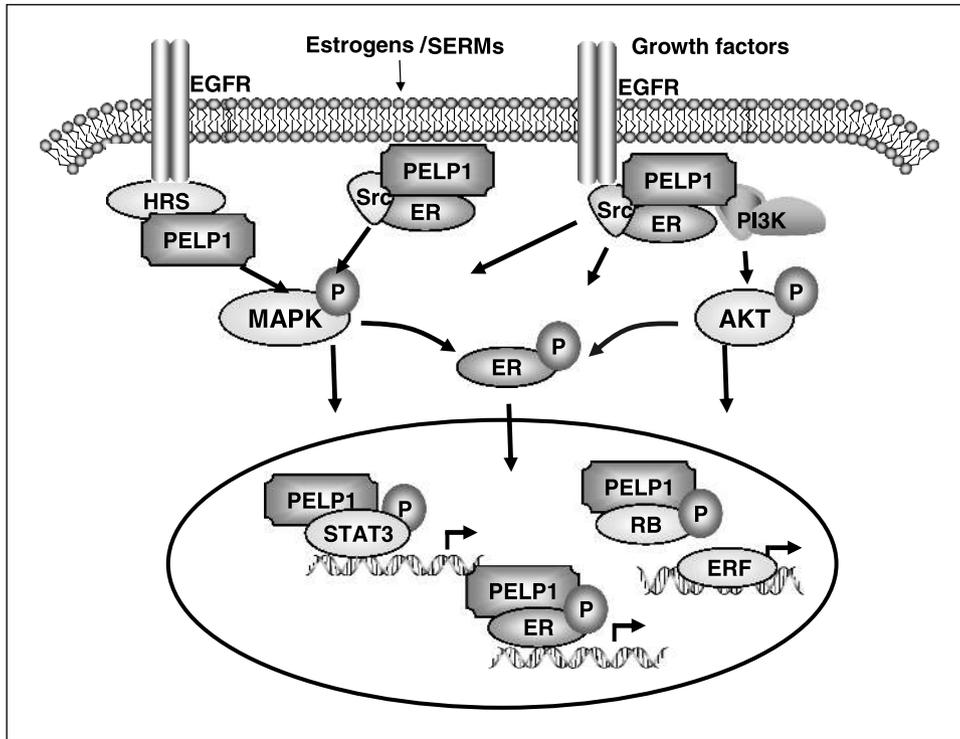
Fig. 2. A schematic model of PELP1/MNAR action defining its role in breast cancer cells, summarizing the cellular events in which PELP1/MNAR has been implicated. PELP1/MNAR can interact with various signaling components as well as ER and is thus involved in the outcomes influenced by ER in the cell (refer to text for a detailed description).

phosphatidylinositol-3-kinase, leading to phosphatidylinositol-3-kinase activation. In addition, PELP1/MNAR interacts with epidermal growth factor receptor and participates in growth factor–mediated ER transactivation functions.

PELP1/MNAR expression and localization are also widely deregulated in endometrial cancers. In addition, PELP1/MNAR and ER were shown to be localized predominantly in the cytoplasm of high-grade endometrial tumors (27). PELP1/MNAR promotes tamoxifen-mediated agonistic action in endometrial model cells (27). Because PELP1/MNAR was primarily localized in either the cytoplasmic or the nuclear compartment in different endometrial cancers, these studies provide important clues about its cytoplasmic (nongenomic) functions. Recent studies have shown that differential compartmentalization of PELP1/MNAR may play a crucial role in modulating the status of nongenomic signaling using molecular mechanisms that remain poorly understood (33). PELP1-cyto cells formed very early and very aggressive tumors compared with those arising from wild-type PELP1 cells (which contained PELP1/MNAR in both the nucleus and the cytoplasm), and these tumors exhibited resistance to tamoxifen. Further characterization of cytoplasmic PELP1/MNAR functions revealed its interaction with a trafficking molecule that activates MAPK in the presence of epidermal growth factor receptor, presumably by sequestering PELP1/MNAR in the cytoplasm. Because MAPK activation is associated with tamoxifen resistance, functional inactivation of the endosomal molecule could potentially sensitize cells to tamoxifen.

PELP1/MNAR is a unique coactivator that plays an important role in both the genomic and nongenomic actions of ER. PELP1/MNAR recruitment to ER target gene chromatin and its interactions with histones, histone acetyltransferase enzymes, and deacetylase components suggest that PELP1/ MNAR participates in chromatin remodeling activity. In addition, the ability of PELP1/MNAR to interact with ER, Src, phosphatidylinositol-3-kinase, Akt, signal transducer and activator of transcription 3, and epidermal growth factor receptor highlights a novel role for PELP1/MNAR in ER nongenomic signaling. Deregulation of PELP1/MNAR localization may help to sequester ER in the cytoplasm/membrane. It is also possible that mislocalization of PELP1/MNAR, which has the potential to stimulate nongenomic ER functions, may alter the ratio of genomic to nongenomic signaling in breast cancer cells and thus might promote hormonal independence by modulating ER-transactivating functions and selective ER modulator actions. Growth factor signaling leading to modification of PELP1/MNAR could be another mechanism promoting hormonal resistance. In addition, in a subset of breast tumors, PELP1/MNAR expression is either lost or undetectable. Because PELP1/MNAR plays an essential role in ER actions, it is tempting to speculate that the absence of PELP1/MNAR could also contribute to resistance to hormonal therapy.

Collectively, these findings suggest that PELP1/MNAR expression or localization could be used to determine whether a tumor will be hormone resistant or susceptible. Drugs targeting PELP1/MNAR or its regulatory pathways (Src and Akt) could be beneficial in those tumors where PELP1/MNAR expression/localization is deregulated. However, these indications of PELP1/MNAR potential are based on a few studies of a small number of tumor samples. Future studies analyzing more tumor samples, validating the role of PELP1/MNAR in animal models of hormone-resistant and hormone-susceptible cancers, and elucidating the mechanisms that cause deregulation of PELP1/MNAR in tumors will help in the development of ways to target this novel gene for endocrine therapy.

MTA Family Members and ER

ER coregulatory proteins have been suggested to play a role in the generally observed tissue-specific effects of tamoxifen (33, 34). However, these ER coregulators are targeted by excessive ER/epidermal growth factor receptor 2 cross talk, leading to hormonal resistance in a subset of breast tumors (35). Growth factor signaling promotes ER phosphorylation on both serine and tyrosine residues (36, 37). Recent evidence suggests that ER coactivators, like ER itself, are targets of growth factor signaling (38). Growth factor–mediated activation of nongenomic pathways and phosphorylation of ER and ER coregulatory proteins have been shown to have a role in tamoxifen resistance (2, 39). MTA1, a presumed corepressor of ER, is a phosphoprotein of which expression can be induced by heregulin (40). Up-regulation of MTA1 is associated with the increased invasiveness and metastatic potential of several human cancers, including carcinomas of the breast and ovaries (41). Inhibition of MTA1 protein expression by antisense phosphorothioate oligonucleotides caused the growth and invasiveness of MDA-MB231 breast cancer cells to be inhibited, which is consistent with MTA1 having a role in metastasis (42). MTA1s (a naturally occurring variant of MTA1) is overexpressed in ER-negative tumors. MTA1s inhibits ER nuclear signaling by sequestering ER in the cytoplasm using its LXXLL motif but enhances ER nongenomic signaling and tumorigenesis (43). These findings suggest a complex role for MTA1 and MTA1s in modulating the hormone-dependent functions of ER.

Future Directions and Perspectives

Endocrine therapy for women with metastatic breast cancer has become more complex than previously envisaged. Several agents that interfere with ER signaling are available clinically. However, the development of resistance is still an unsolved problem and is addressed only by using these drugs in different "endocrine cascades." Current insights into the molecular basis for resistance are rudimentary but are most clearly illuminated by investigations that focus on the cross-talk between receptor tyrosine kinases and ER. However, in some tumors, endocrine resistance occurs in the absence of any evidence of receptor tyrosine kinase overexpression. In these instances, common downstream signal transduction proteins that are known to intersect with the ER pathway, such as Pak1 (and its targets), provide a potential therapeutic target to address resistance. The tissue- and cell type–specific effects of estrogen have been attributed largely to the different coregulators and specific promoters that ER associates with, in addition to the various ER subtypes and extranuclear signaling events. Recent investigations have proved conclusively that signaling to ER or its coregulators is a key factor contributing to resistance. Thus, in addition to ER itself, ER coregulators are potential clinical intervention targets.

Although much information has been learned in the past three decades about ER and cancer, a lot more remains to be learned. The establishment of mouse and cell lines models that accurately mimic characteristics of estrogen independence and the biochemistry of human breast cancers would greatly advance the field. Preclinical investigations using such models might lead to a more efficient design of clinical trials by predicting treatment effects and their mechanisms of action.

Open Discussion

Dr. Myles Brown: If we identify multiple *possible* mechanisms of resistance, the issue then becomes what are the *actual* mechanisms of resistance in patients. This issue points to something I have been advocating, which is the need to identify in the patient's tissue at the time of relapse which pathways are involved in resistance.

Dr. Kumar: This is a major challenge to all of us. I can only tell you that we are over 150 cases of hormone-responsive endometrial, salivary gland, and breast cancers now, and I would say that in a significant number of cases, PELP1 was in cytoplasm. I hope that we can put together a consortium to go from the mouse or the human material and ask that question in a focused manner. Many of these studies are limited in terms of a reasonable correlation with the human material and data from tissue culture. But, again, we will learn a great deal about the biology, which is one of the key purposes of such preclinical model systems. We really have no idea at the moment what will be significance of the coactivators or ER modification in relation to antiestrogen action in human breast tumors. We have to go back to the tumors and then try to mimic a model system, study the model system, and then go back again to the tissue. This is not going to be an easy task, and no single lab can do it.

Dr. Mitch Dowsett: Getting and studying tissue at the time of recurrence is something we would all like to do, but it is going to continue to be tough. But I think there are valuable analogous data in primary breast cancers at presentation. One can assess these phenotypic characteristics in relation to changes in Ki67, for example. This is a rather easier situation to work with. Therefore, looking at these pathways at that level of the primary tumor may well give us an entrée into what is happening in recurrent disease.

Dr. Kumar: When we think about the components of the ER pathway, we want only to link these components with the ER. But there are components of the ER pathway that may not be dependent on ER itself. We have identified some of these components using a yeast two-hybrid screening for PELP1 or ER as baits. A few of these new proteins may be responsible for cross-talk and perhaps are mediators of growth factor receptor signaling. Of course, ER response is the goal, but that is not the only way to look at it. You will be seeing some papers from my and Ratna Vadlamudi's labs and from others where PELP is likely to be involved in signaling other than ER.

Dr. Brown: The evidence supporting a role for a nuclear chromatin-bound ER and for a cytoplasmic ER that receives and sends signals is all extremely strong. I just wonder what your thoughts are about the role for a lipid-modified nuclear receptor ERα getting into the membrane—whether you think that has really been demonstrated and whether you think it might play a significant role in estrogen response or not?

Dr. Kumar: I believe ER can be found in close proximity to the plasma membrane, but it may not be in the membrane. In fact, if you knock down caveolin by small interfering RNA, you hardly see any major change in ER location. In these studies, we have failed to clearly see any major change in the ER localization in our lab. Our own feeling is that membrane ER

may or may not be a major player, though it will be very important for delineation of the ER signaling pathway. But, really, we are only talking about a very small fraction of total ER. What is really important is to define all kinds of modifications or demodifications to which ER protein might be subject in the cytoplasm. If you do a careful screen, you'll be surprised to see how many of these demodifying or modifying enzymes are pulling down with a different component of ER such as PELP1.

Dr. Adrian Lee: We spent the last 2 or 3 years trying to put ER in the membrane. It is easy to put it in the cytoplasm, though it doesn't like to go there. So if there is going to be some ER there in the membrane, it is going to be a small amount. Until we have models without nuclear ER and with the whole receptor in the membrane, I think it will be hard to address these questions.

Dr. Brown: All the evidence for an integral membrane form of ER, or a lipid-modified form in the membrane, is extremely weak. Yet the concept has gained acceptance. In reviews and in model slides, the figures are all drawn now with ER shooting into the membrane. Given its crystal structure, I would challenge anybody to show me the biochemistry that would allow ER itself, unless it is lipid modified, to be within the membrane.

Dr. Kumar: One of the problems in attempting to detect membrane ER, or membrane-bound ER, is that we are still lacking reagents that can selectively pick up a membrane form of ER. Overall, the current evidence is not derived from a physiologically relevant setting.

Dr. Brown: I'm willing to be convinced that there is a specific form of ER that is bound to proteins that anchor to the membrane, but the evidence isn't there.

Dr. Kumar: We have found a specific modification of ER. If we use the ER antibody, we are not detecting anything outside the nucleus and have a lot of noise. So we are now using a specific antibody to this modification. We are getting a major change in the signaling now. The noise has almost gone, and we now are seeing some ER close to the membrane. But again, it may or may not be membrane bound. One way to address this issue is to have specific antibodies to detect only these modifications. Then you will start seeing the evidence. But right now we are not there.

Acknowledgments

References

1. Buzdar AU. Endocrine therapy in the treatment of metastatic breast cancer. Semin Oncol 2001;28: 291–304.
2. Clarke R, Leonessa F, Welch JN, Skaar TC. Cellular and molecular pharmacology of antiestrogen action and resistance. Pharmacol Rev 2001;53:25–71.
3. Hoffmann J, Sommer A. Steroid hormone receptors as targets for the therapy of breast and prostate cancer—recent advances, mechanisms of resistance, and new approaches. J Steroid Biochem Mol Biol 2005;93:191–200.
4. Nicholson RI, Hutcheson IR, Knowlden JM, et al. Nonendocrine pathways and endocrine resistance: observations with antiestrogens and signal transduction inhibitors in combination. Clin Cancer Res 2004; 10:346–54S.
5. Levin ER. Integration of the extranuclear and nuclear actions of estrogen. Mol Endocrinol 2005;19:1951–9.
6. Bjornstrom L, Sjoberg M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol Endocrinol 2005;19:833–42.
7. Acconcia F, Kumar R. Signaling regulation of genomic and nongenomic functions of estrogen receptors. Cancer Lett. In press 2005.
8. Michalides R, Griekspoor A, Balkenende A, et al. Tamoxifen resistance by a conformational arrest of the estrogen receptor α after PKA activation in breast cancer. Cancer Cell 2004;5:597–605.
9. Revelli A, Massobrio M, Tesarik J. Nongenomic actions of steroid hormones in reproductive tissues. Endocr Rev 1998;19:3–17.
10. Schiff R, Massarweh S, Shou J, Osborne CK. Breast cancer endocrine resistance: how growth factor signaling and estrogen receptor coregulators modulate response. Clin Cancer Res 2003;9:447–54S.
11. Graham JD, Bain DL, Richer JK, Jackson TA, Tung L, Horwitz KB. Nuclear receptor conformation, coregulators, and tamoxifen-resistant breast cancer. Steroids 2000;65:579–84.
12. Shou J, Massarweh S, Osborne CK, et al. Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. J Natl Cancer Inst 2004;96:926–35.
13. Adam L, Vadlamudi R, Mandal M, Chernoff J, Kumar R. Regulation of microfilament reorganization

and invasiveness of breast cancer cells by kinase dead p21-activated kinase-1. J Biol Chem 2000;275: 12041–50.
14. Vadlamudi RK, Adam L, Wang RA, et al. Regulatable expression of p21-activated kinase-1 promotes anchorage-independent growth and abnormal organization of mitotic spindles in human epithelial breast cancer cells. J Biol Chem 2000;275:36238–44.
15. Bagheri-Yarmand R, Mandal M, Taludker AH, et al. Etk/Bmx tyrosine kinase activates Pak1 and regulates tumorigenicity of breast cancer cells. J Biol Chem 2001;276:29403–9.
16. Kumar R, Vadlamudi RK. Emerging functions of p21-activated kinases in human cancer cells. J Cell Physiol 2002;193:133–44.
17. Bokoch GM. Biology of the p21-activated kinases. Annu Rev Biochem 2003;72:743–81.
18. Wang RA, Mazumdar A, Vadlamudi RK, Kumar R. P21-activated kinase-1 phosphorylates and transactivates estrogen receptor-α and promotes hyperplasia in mammary epithelium. EMBO J 2002;21:5437–47.
19. Balasenthil S, Sahin AA, Barnes CJ, et al. p21-activated kinase-1 signaling mediates cyclin D1 expression in mammary epithelial and cancer cells. J Biol Chem 2004;279:1422–8.
20. Vadlamudi RK, Bagheri-Yarmand R, Yang Z, et al. Dynein light chain 1, a p21-activated kinase 1-interacting substrate, promotes cancerous phenotypes. Cancer Cell 2004;5:575–85.
21. Rayala SK, den Hollander P, Balasenthil S, Yang ZB, Broaddus RR, Kumar R. Functional regulation of oestrogen receptor pathway by the dynein light chain 1. EMBO Rep 2005;6:538–44.
22. Vadlamudi RK, Wang RA, Mazumdar A, et al. Molecular cloning and characterization of PELP1, a novel human coregulator of estrogen receptor α. J Biol Chem 2001;276:38272–9.
23. Wong CW, McNally C, Nickbarg E, Komm BS, Cheskis BJ. Estrogen receptor-interacting protein that modulates its nongenomic activity-crosstalk with Src/Erk phosphorylation cascade. Proc Natl Acad Sci U S A 2002;99:14783–8.
24. Joung I, Strominger JL, Shin J. Molecular cloning of a phospho tyrosine-independent ligand of the p56lck SH2 domain. Proc Natl Acad Sci U S A 1996;93: 5991–5.

25. Balasenthil S, Vadlamudi RK. Functional interactions between the estrogen receptor coactivator PELP1/MNAR and retinoblastoma protein. J Biol Chem 2003; 278:22119–27.
26. Pawlak J, Beyer C. Developmental expression of MNAR mRNA in the mouse brain. Cell Tissue Res 2005;320:545–9.
27. Vadlamudi RK, Balasenthil S, Broaddus RR, Gustafsson JA, Kumar R. Deregulation of estrogen receptor coactivator proline-, glutamic acid-, and leucine-rich protein-1/modulator of nongenomic activity of estrogen receptor in human endometrial tumors. J Clin Endocrinol Metab 2004;89:6130–8.
28. Barletta F, Wong CW, McNally C, Komm BS, Katzenellenbogen B, Cheskis BJ. Characterization of the interactions of estrogen receptor and MNAR in the activation of cSrc. Mol Endocrinol 2004;18:1096–108.
29. Nair SS, Mishra SK, Yang Z, Balasenthil S, Kumar R, Vadlamudi RK. Potential role of a novel transcriptional coactivator PELP1 in histone H1 displacement in cancer cells. Cancer Res 2004;64:6416–23.
30. Choi YB, Ko JK, Shin J. The transcriptional corepressor, PELP1, recruits HDAC2 and masks histones using two separate domains. J Biol Chem 2004; 279:50930–41.
31. Mishra SK, Talukder AH, Gururaj AE, et al. Upstream determinants of estrogen receptor-α regulation of metastatic tumor antigen 3 pathway. J Biol Chem 2004;279:32709–15.
32. Manavathi B, Nair S, Wang R, Kumar R, Vadlamudi R. Proline glutamic acid and leucine rich protein-1 is essential in growth factor regulation of signal transducers and activators of transcription 3 activation. Cancer Res 2005;65:5571–7.
33. Vadlamudi RK, Manavati B, Balasenthil S, Nair S, Yang Z, Kumar R. Functional implications of altered subcellular localization of PELP1 in breast cancer cells. Cancer Res 2005;65:7724–32.
34. Haas D, White SN, Lutz LB, Rasar M, Hammes SR. The modulator of nongenomic actions of the estrogen receptor (MNAR) regulates transcription-independent androgen receptor-mediated signaling: Evidence that MNAR participates in G protein-regulated meiosis in *Xenopus laevis* oocytes. Mol Endocrinol 2005;19: 2035–46.
35. Unni E, Sun S, Nan B, et al. Changes in androgen

receptor nongenotropic signaling correlate with transition of LNCaP cells to androgen independence. Cancer Res 2004;64:7156 – 68.

36. Pearce ST, Jordan VC. The biological role of estrogen receptors α and β in cancer. Crit Rev Oncol Hematol 2004;50:3 – 22.

37. Arnold SF, Obourn JD, Jaffe H, Notides AC. Phosphorylation of the human estrogen receptor on tyrosine 537 *in vivo* and by src family tyrosine kinases *in vitro*. Mol Endocrinol 1995;9:24 – 33.

38. Wu RC, Qin J, Yi P, et al. Selective phosphorylations of the SRC-3/AIB1 coactivator integrate genomic reponses to multiple cellular signaling pathways. Mol Cell 2004;15:937 – 49.

39. Hutcheson IR, Knowlden JM, Madden TA, et al. Oestrogen receptor-mediated modulation of the EGFR/MAPK pathway in tamoxifen-resistant MCF-7 cells. Breast Cancer Res Treat 2003;81:81 – 93.

40. Mazumdar A, Wang RA, Mishra SK, et al. Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor. Nat Cell Biol 2001;3:30 – 7.

41. Nicolson GL, Nawa A, Toh Y, Taniguchi S, Nishimori K, Moustafa A. Tumor metastasis-associated human MTA1 gene and its MTA1 protein product: role in epithelial cancer cell invasion, proliferation and nuclear regulation. Clin Exp Metastasis 2003;20:19 – 24.

42. Nawa A, Nishimori K, Lin P, et al. Tumor metastasis-associated human MTA1 gene: its deduced protein sequence, localization, and association with breast cancer cell proliferation using antisense phosphorothioate oligonucleotides. J Cell Biochem 2000;79:202 – 12.

43. Kumar R, Wang RA, Mazumdar A, et al. A naturally occurring MTA1 variant sequesters oestrogen receptor-α in the cytoplasm. Nature 2002;418:654 – 7.

Clinical Cancer Research

Novel Mechanisms of Resistance to Endocrine Therapy: Genomic and Nongenomic Considerations

Anupama E. Gururaj, Suresh K. Rayala, Ratna K. Vadlamudi, et al.

Updated version	Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/12/3/1001s

Cited articles	This article cites 42 articles, 17 of which you can access for free at: http://clincancerres.aacrjournals.org/content/12/3/1001s.full#ref-list-1
Citing articles	This article has been cited by 11 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/12/3/1001s.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/12/3/1001s. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.